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GERM-CELL DETERMINANTS AND THEIR SIGNIFICANCE[1]

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INVESTIGATIONS of the origin of the germ cells in a number of animals have brought forth certain phenomena which indicate that these cells are determined as such at a very early period in embryonic development, and that in some cases the material which apparently determines the germ cells is visible at this time. Conclusions can be drawn from these observations which are of considerable interest.

The frequently repeated statement that the germ cells are derived from the mesoderm or from the entoderm is of course erroneous in those instances where the germ cells can be identified before the formation of the germ layers, and it seems probable that the primary cell differentiation, *i. e.*, the separation of the germ cells from the somatic cells, takes place at an early period in the embryonic development of even those animals where this has not been actually observed. A few of the most pronounced cases of the early differentiation of germ cells are briefly described in the following paragraphs and several general conclusions arrived at from this evidence.

The best known example is *Ascaris*, as described by

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Boveri ('92). The first cleavage division of the egg of *Ascaris* results in two daughter cells, each containing two long chromosomes (Fig. 1, *A*). In the second division the chromosomes of one cell divide normally and each daughter cell receives one half of each (Fig. 1, *B, s*). The chromosomes of the other cell behave differently; the thin middle portion of each breaks up into granules

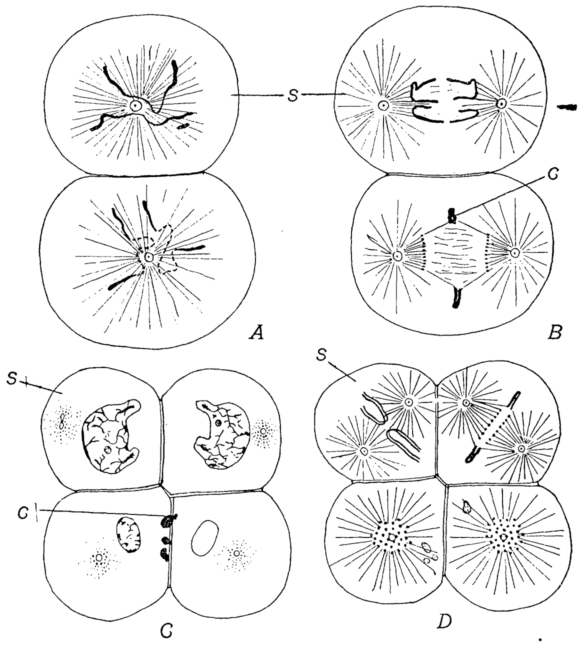

FIG. 1. Origin of the primordial germ cells and casting out of chromatin in the somatic cells of *Ascaris*. (From Wilson's cell, after Boveri.) *A*, two-cell stage dividing; *s*, stem-cell, from which arise the germ cells. *B*, the same from the side, later in the second cleavage, showing the two types of mitosis and the casting out of chromatin (*c*) in the somatic cell. *C*, resulting four-cell stage; the eliminated chromatin at *c*. *D*, the third cleavage, repeating the foregoing process in the two upper cells.

(Fig. 1, *A*) which split, half going to each daughter cell, but the swollen ends (Fig. 1, *B, c*) are cast off into the cytoplasm. In the four-cell stage there are consequently two cells with the full amount of chromatin and two with a reduced amount. This inequality in the amount of chromatin results in different sized nuclei (Fig. 1, *C*); those with entire chromosomes (*s*) are larger than those that have lost the swollen ends (*c*). In the third division

one of the two cells with the two entire chromosomes loses the swollen ends of each; the other (Fig. 1, *D*, *s*) retains its chromosomes intact. A similar reduction in the amount of chromatin takes place in the fourth and fifth divisions and then ceases. The single cell in the thirty-two-cell stage which contains the full amount of chromatin has a larger nucleus than the other thirty-one cells and gives rise to all of the germ cell, whereas the other cells are for the production of somatic cells only.

The primordial germ cell of *Ascaris*, therefore, contains two entire chromosomes; every other cell possesses two chromosomes which have lost part of their substance. In other words, the germ cells possess a certain amount of chromatic material not present in the somatic cells.

There is also an early differentiation of the germ cells in the fresh water crustacean, *Cyclops* (Haecker, '97). According to Haecker, "Aussenkörnchen" arise at one pole of the first cleavage spindle (Fig. 2, *A*, *ak*); these

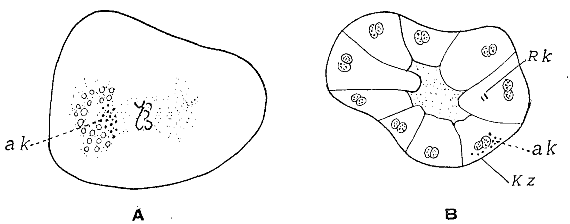

FIG. 2. Origin of the primordial germ cell in *Cyclops*. (From Haecker.) *A*, the first cleavage division, showing the "Aussenkörnchen" (*ak*) at one pole of the spindle. *B*, the thirty-two-cell stage; the primordial germ cell (*Kz*) contains all of the "Aussenkörnchen" (*ak*).

are derived from disintegrated nucleolar material and are attracted to one pole of the spindle by a dissimilar influence of the centrosomes. During the first four cleavage divisions the granules are segregated always in one cell (Fig. 2, *B*, *kg*); at the end of the fourth division these "Aussenkörnchen" disappear, but the cell which contained them can be traced by its delayed mitotic phase and is shown to be the primordial germ cell.

In this case, as in that of *Ascaris*, the primordial germ cell and the germ cells derived from it possess certain nuclear materials not present in the somatic cells. The

latter seem to be limited because of their absence to the performance of vegetative functions, and the germ cells appear to have the power of reproduction because of their presence.

A recent paper by Elpatiewsky (1909) deals with the early embryonic development of the arrow worm, *Sagitta*. This investigator finds that, at the stage when the two pronuclei are in the center of the egg, a body appears at the vegetative pole lying near the periphery (Fig. 3,

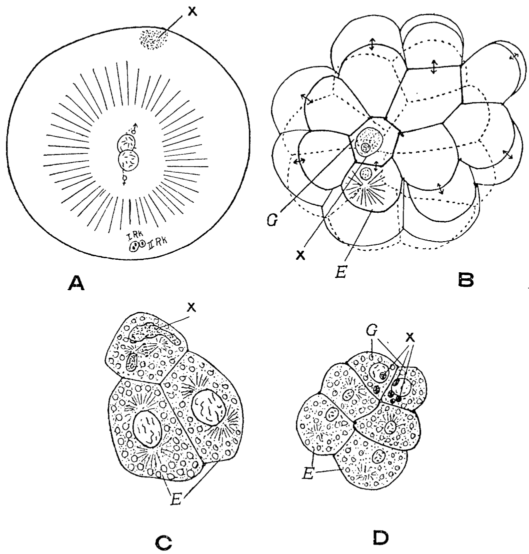

FIG. 3. Origin of the primordial germ cells in *Sagitta*. (From Elpatiewsky.) *A*, egg with conjugating pronuclei; polar bodies (*I Rk.* and *II Rk.*) and "besonderer körper" (x) embedded in cytoplasm. *B*, thirty-two-cell stage; the primordial germ cell (G) contains the "besonderer körper" (x); entoderm cell at E. *C*, the primordial germ cell dividing; the "besonderer körper" (x) within it is also dividing; the entoderm cell has already divided (E). *D*, two germ cells (G) resulting from the first division of the primordial germ cell; each contains part of the "besonderer körper" (x).

A). This body, which is termed "besondere körper," consists of coarse granules which do not stain quite so deeply as the chromosomes. During the first four cleavage divisions the "besondere körper" does not divide, but is always to be found in one blastomere. In the fourth division the blastomere which contains this body divides unequally; the larger cell is destined to produce the entoderm (Fig. 3, *B*, *E*); the smaller cell, which con-

tains the "besondere körper" is the primordial germ cell (Fig. 3, *B, G*). The first division of this primordial germ cell (Fig. 3, *C*) results in two daughter cells, one of which obtains a larger portion of the "besondere körper" (*X*) than the other (Fig. 3, *D*). This is interpreted as the differential division, the cell which possesses the larger amount of the divided "besondere körper" giving rise to the male germ cells, the other to the female germ cells in the hermaphroditic adult. The "besondere körper" now gradually becomes paler and finally disappears. Buchner ('10) and Stevens ('10) have confirmed Elpatiewsky's observations. The origin of the "besondere körper" was not determined.

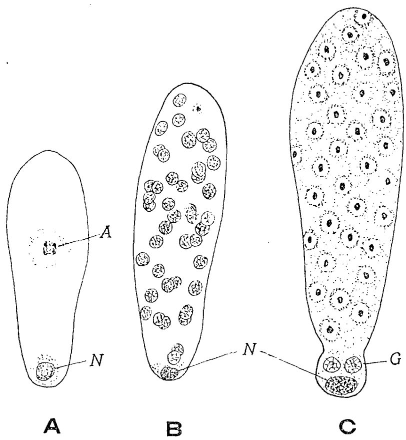

FIG. 4. Origin of the primordial germ cells of *Oophthora*. (From Silvestri.) *A*, the nucleolus (*N*) of the germinal vesicle (*A*) has passed to the posterior end of the egg. *B*, an egg containing a number of cleavage nuclei. *C*, the cells which come under the influence of the nucleolus (*N*) become the primordial germ cells (*G*).

These investigations show the germ cells of *Sagitta* to be similar to those of *Ascaris* and *Cyclops* in that they contain a darkly staining material not present in the somatic cells.

In *Oophthora* and other parasitic hymenoptera, Sylvestri ('09) finds that the nucleolus of the germinal vesicle passes to the posterior end of the egg during maturation (Fig. 4, *A*). Here it remains until the cleavage nuclei reach the periphery (Fig. 4, *B*). The cells which then come under the influence of the nucleolus be-

come the primordial germ cells (Fig. 4, *C, G*) and give rise to the germ glands of the adult. The similarity between this process and that described for *Ascaris, Cyclops* and *Sagitta* is obvious.

Finally in chrysomelid beetles the primordial germ cells are differentiated at a very early period (Hegner, '09). At the posterior end of the eggs of *Calligrapha multipunctata* and allied species there is a disc-shaped mass of granules which stain like chromatin. I have called this the pole disc (Fig. 5, *A, gc. d*). When the cleavage nuclei reach the periphery of the egg they fuse with the superficial layer of cytoplasm everywhere except at the posterior end; cell walls then appear and a blastoderm is formed. When the cleavage nuclei which reach the posterior end of the egg encounter the pole disc granules they gather these granules about them and continue their migration (Fig. 5, *B, gc*); cell walls are formed, and they finally come to lie entirely outside of the egg (Fig. 5, *C, gc*).

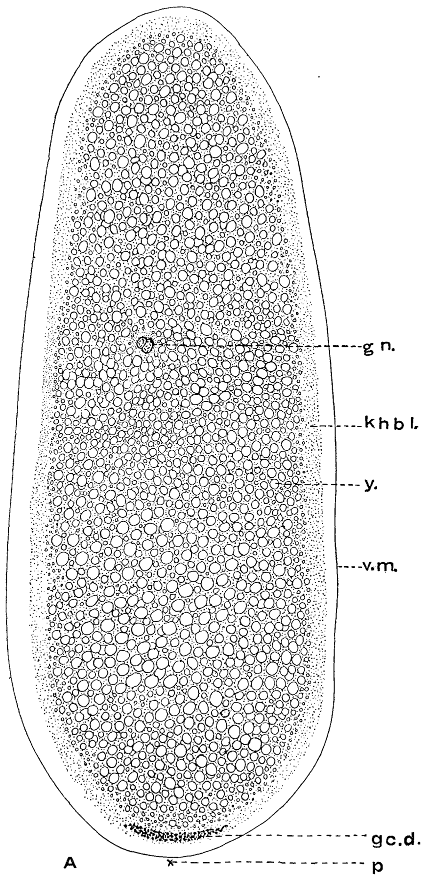

FIG. 5.

There are sixteen cells which separate from the egg in this manner, and they take out of the egg with them practically all of the pole disc granules (Fig. 5, *C, pd.g*). These sixteen cells divide to form thirty-two; in this division apparently one half of the granules contained in each cell pass to each of the daughter cells (Fig.

5, *D, pd.g*). A second division results in sixty-four cells; this number is constant until a late stage in embryonic development.

These sixty-four cells have been traced through the early embryonic stages. First they migrate back into the egg through a "pole cell canal" (Fig. 5, *C, pc*) sit-

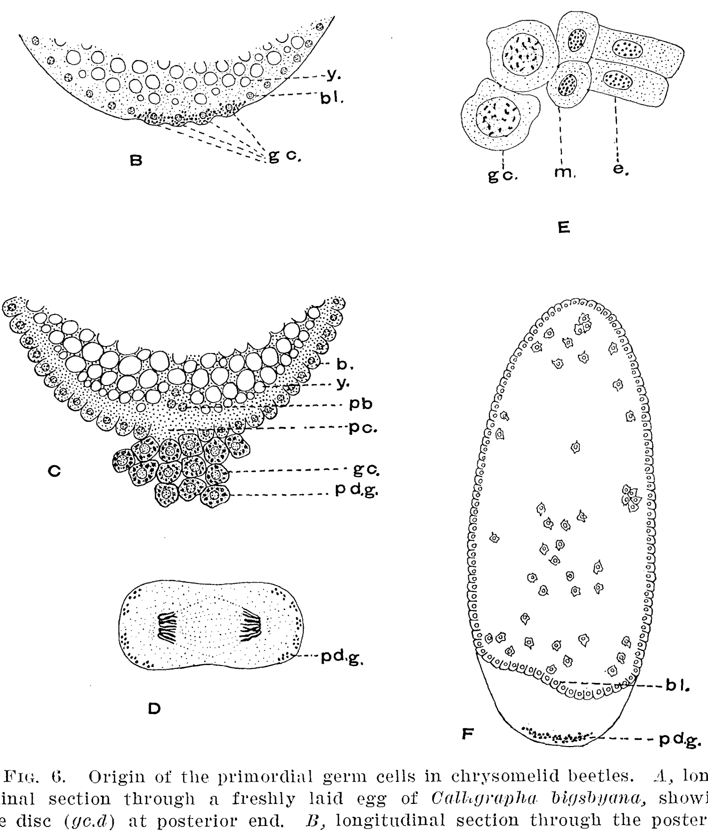

FIG. 6. Origin of the primordial germ cells in chrysomelid beetles. *A*, longitudinal section through a freshly laid egg of *Calligrapha bigsbyana*, showing pole disc (*gc.d*) at posterior end. *B*, longitudinal section through the posterior end of an egg of *C. multipunctata* eighteen hours after deposition, showing pole disc granules within the primordial germ cells (*gc*). *C*, longitudinal section through the posterior end of an egg of *C. bigsbyana* twenty-four hours after deposition, showing the primordial germ cells containing pole disc granules (*pd.g*). *D*, a primordial germ cell of *C. multipunctata* in anaphase of mitosis; the pole disc granules (*pd.g*) are apparently equally distributed at either end. *E*, two germ cells (*gc*) and neighboring mesoderm cells (*m*) and ectoderm cells (*e*) from an embryo of *C. multipunctata*. *F*, longitudinal section through an egg of *Leptinotarsa decemlineata* twenty-four hours after the posterior end had been killed with a hot needle, thus preventing the pole disc granules (*pd.g*) from taking part in the development. No germ cells are formed (compare with *C*). *bl* = blastoderm; *e* = ectoderm cell; *gc* = germ cell; *gc.d* = germ cell determinants; *gn* = conjugating pronuclei; *khbl* = keimhautblastem; *m* = mesoderm cell; *p* = posterior end of egg; *pbl* = pseudoblastodermic nuclei; *p.c* = pole cell canal; *pd.g* = pole disc granules; *vm* = vitelline membrane; *y* = yolk.

uated near the posterior end of the ventral groove. Then they separate into two apparently equal groups, one on either side of the embryo, which are soon recognizable as the two germ glands.

Because only those cells which gather in the pole disc granules become germ cells, I have called these granules "germ cell determinants" (Hegner, '08). This term has been objected to by Wieman ('10) because "the term implies the attribute of certain potentialities that these granules have not been shown to possess" (p. 180). The morphological evidence is, I believe, strong enough to warrant the use of the term; recent experiments, however, add to the convincing facts already published (Hegner, '08, '09). It is possible to show that if the pole disc is prevented from taking part in the development of the egg, no germ cells will be produced. Attempts to extract the pole disc by means of pricking the freshly laid egg and allowing them to flow out were only partially successful (Hegner, '08). A new method was later employed which absolutely prevented the cleavage nuclei from encountering the pole disc. In these experiments the posterior end of the egg was touched with a hot needle and that portion containing the pole disc was killed. In every instance the development continued and in the eggs so far examined the blastoderm formed normally over all of the surface except at the posterior end; here it was built at the end of the living substance as shown in Fig. 5, *F, bl.* No germ cells were produced. I conclude from this that the pole disc granules *are* necessary for the formation of germ cells, and that they are really "germ cell determinants." Of course it might be argued that some other substance lying at the posterior end of the egg is responsible for the differentiation of the germ cells, but this seems highly improbable. Wieman ('10) states that in *Leptinotarsa signaticollis*, a species I have not studied, "the granules are not all taken up by the cells in their migration and the greater part of them remains behind after the cells have passed through" (p. 186). This is certainly not the case in the many eggs that I have examined, and a reexamination

shows that only a few of the pole disc granules remain in the egg after the germ cells are formed, as was clearly pointed out in a former paper (Hegner, '09, Plate II, Fig. 16).

The origin of the pole disc granules is not known. It seemed to me probable that they came from the nucleus of the egg just before maturation and consisted of nuclear material. This conclusion was reached (1) because these granules stain like chromatin, (2) because in many insects the nucleus of the oogonium casts out chromatic material (Nebenkerne), and (3) because the substance which determines the germ cells in *Ascaris, Cyclops* and *Oophthora* is of nuclear origin, and in one case (*Ascaris*) is chromatin. Wieman believes that "the granules of the pole disc consist of particles derived from the food stream of the ovum that form an accumulation in the protoplasm in its posterior part" (p. 187). This possibility was pointed out in a former paper (Hegner, '09, p. 274), a fact Wieman seems to have overlooked. It was also suggested in the same place that if the granules are derived from the nurse cells they probably come from the nuclei of these cells. The pole disc granules gradually disappear after the germ cells are formed.

It may be of interest to mention the results of operations performed upon eggs in which the germ cells had already differentiated at the posterior end (Fig. 5, *C*). Such eggs, when touched with a hot needle, continued to develop, and produced embryos and larvæ without germ glands. This I believe is the earliest stage on record on which surgical castration has been performed.

The visible presence of germ cell determinants in the primordial germ cells of the animals described above suggests two possibilities as to their importance: (1) They may represent idiochromatin, *i. e.*, germ plasm, or (2) they may influence the metabolism of the cells and thus determine their character.

1. The history of the germ cells in chrysomelid beetles illustrates in a remarkable way the theory of germinal continuity as expressed by Weismann ('04). Weismann believes with Nägeli that "there are two great categories

of living substance—hereditary substance or idioplasm, and 'nutritive substance' or trophoplasm, and that the former is much smaller in amount than the latter'' (Weismann, '04, Vol. I, p. 341). The idioplasm of the germ cells he calls germ plasm, a substance which is ''never formed *de novo,* but it grows and increases ceaselessly; it is handed on from one generation to another like a long root creeping through the earth, from which at regular distances shoots grow up and become plants, the individuals of the successive generations'' (Vol. I, p. 416). ''This splitting up of the substance of the ovum into a somatic half, which directs the development of the individual, and a propagative half which reaches the germ cells and there remains inactive, and later gives rise to the succeeding generation, constitutes *the theory of the continuity of the germ plasm* (Vol. I, p. 411). According to this theory, the body or somatic cells serve only to protect, nourish and transport the germ cells which contain the germ plasm. Later the germ cells separate from the body and develop into new individuals and the body subsequently dies.

In the eggs of chrysomelid beetles the germ cells are formed at an extremely early period in embryonic development. They separate entirely from the embryo and come to lie in a group at the posterior end; at this time germ cells are quite distinct from somatic cells. Later the germ cells migrate back into the embryo, where they are protected, nourished and transported until they become mature, leave the body and give rise to a new generation.

What particular part of the germ cell represents the idioplasm or germ plasm? is a question of fundamental importance. Weismann recognizes the chromosomes as the germ plasm and has built up a complex theory as to the constituents of these bodies. The present discussion is not concerned in any way with the structure of the germ plasm as conceived by Weismann, and the writer does not wish to become involved in a consideration of idants, ids, determinants and biophores. The theory of dichromaticity (Dobell, '09) may aid in answering this

question. This theory holds that the chromatin of the germ cells is of two kinds—(1) idiochromatin, which is for reproductive purposes, and (2) trophochromatin which performs vegetative functions. In many Protozoa these two kinds of chromatin are separate throughout the life cycle. For example, in *Paramecium* the micronucleus is thought to represent the idiochromatin, the macronucleus, the trophochromatin (Calkins, '09). During conjugation and the subsequent reörganization of the nuclear apparatus the macronucleus breaks down and disappears, whereas the micronucleus gives rise not only to new bodies like itself, but also to new macronuclei.

In most animals idiochromatin and trophochromatin are supposed to be contained in one nucleus and are indistinguishable except in a few cases during the differentiation of the germ cells at an early developmental period of the egg. One is tempted to interpret as idiochromatin (1) that part of the chromosomes of *Ascaris* which is lost by the somatic cells (Fig. 1, *B, c*) but retained by the germ cells, (2) the nuclear material which is present in the primordial germ cell of *Cyclops* (Fig. 2, *B, ak*) but is absent from the somatic cells, (3) the similar substance in the primordial germ cells of *Oophthora* (Fig. 4, *n*), (4) the "besondere körper" in the egg of *Sagitta* (Fig. 3, *x*), and (5) the pole disc in the eggs of chrysomelid beetles (Fig. 5, *A, gc.d*).

One difference between these substances and the germ plasm as Weismann conceives it should be pointed out. In the cases cited above the material interpreted as germ plasm is only in one instance chromatin, and in this animal (*Ascaris*) it does not constitute the entire chromatin as Weismann's theory demands. If these extra nuclear bodies really represent the iodioplasm our location of the germ plasm must be transferred from the chromosomes to this material.

2. The second theory mentioned above, namely, that the extra material possessed by the germ cells determines these as such because of some fundamental principle of metabolism, seems more plausible than the theory just outlined. It is worthy of note that the primordial germ

cells of several animals belonging to widely separated groups are supplied with extra nutritive material. This is true in the Diptera, *Chironomus* (Weismann, '63) and *Simula* (Metschnikoff, '66), in the Lepidopteron, *Endromis* (Schwangart, '05), in the Elasmobranchs (Beard, '02), in the Teleosts (Eigenman, '92), in the Amphibia (Nussbaum, '80), and in the Reptilia (Allen, '06).

It has already been pointed out (Hegner, '09) that the pole disc granules may be nutritive material. "That the pole-cells need special means of nourishment is doubtless the case, for contrary to the condition in the blastoderm cells, they are at an early period entirely separated from the yolk, and later use up energy in their migration" (p. 275). If this is true, and as Wieman ('10) claims, the pole disc granules are derived from the yolk stream, our germ cell determinant hypothesis is not weakened, but gains a distinct argument in its favor.

It is interesting to note in this connection that two of the foremost investigators of the relation of the accessory chromosomes to sex determination are inclined to believe in the quantitative hypothesis, *i. e.*, that the egg which is fertilized by the spermatozoon containing the accessory develops into a female because there is more chromatin present, and that this plus amount influences the metabolism of the cell and its descendants (Wilson, '10; Morgan, '10). This hypothesis suggests the theory of sex advocated by Geddes and Thomson ('89), that "the female is the outcome and expression of preponderant anabolism, and in contrast the male of preponderant katabolism" (p. 132). In *Sagitta* (Elpatiewsky, '09), however, it is the male primordial germ cell and not the female that acquires the larger part of the "besondere körper."

Although neither of the two possibilities advanced in the foregoing pages may be correct, nevertheless it seems certain that the peculiar bodies in the primordial germ cells of the animals described above should be named "germ cell determinants." In any event, the attention of investigators ought to be directed toward the problem of discovering the origin and complete history of these

bodies, since their bearing upon theories of heredity is of fundamental importance.

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